IN THE CLAIMS

The claims are as follows:

1. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction which is not a beetle luciferase-mediated reaction; and

(b) introducing a composition capable of selectively quenching the first enzyme-mediated luminescence reaction and initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction, wherein the composition comprises at least one selective quench reagent for the first enzyme-mediated luminescence reaction; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

2. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction; and

(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing a composition comprising a colored compound to the luminescence reaction which compound is a selective quench reagent.

3. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction which is not a beetle luciferase-mediated reaction; and

(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing a composition comprising at least one selective quench reagent to the luminescence reaction;

(c) introducing a composition capable of initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction; and

(d) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

4. (Original) The method according to claim 2 in which the composition further comprises reagents capable of initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

5. (Original) The method according to claim 1 or 3 wherein at least one selective quench reagent is a substrate analog inhibitor for the first enzyme.

6. (Original) The method according to claim 1 or 3 wherein at least one selective quench reagent is a sequestering agent.

7. (Original) The method according to claim 6 wherein the sequestering agent sequesters a substrate for the first enzyme but not the second enzyme.

8. (Original) The method according to claim 6 wherein the sequestering agent is a nonionic detergent.

9. (Original) The method according to claim 6 wherein the sequestering agent is a crown ether, glycol, or cyclodextran.

10. (Original) The method according to claim 1 or 3 wherein at least one selective quench reagent is a colored compound.

11. (Original) The method according to claim 10 wherein the colored compound quenches blue, green or red light.

12. (Original) The method according to claim 1 or 3 wherein in step (a), a luciferase-mediated luminescence reaction is detected or determined.

13. (Original) The method according to claim 12 wherein the luciferase-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof.

14. (Original) The method according to claim 12 wherein in step (b), the first enzyme-mediated reaction is quenched with a nonionic detergent which is not Triton® X-100 or Tween® 20, a substrate analog inhibitor which is a protected coelenterazine, a yellow compound, or a combination thereof.

15. (Original) The method according to claim 14 wherein the luciferase-mediated luminescence reaction is mediated by *Renilla reniformis* (sea pansy) luciferase or a functional equivalent thereof.

16. (Original) The method according to claim 2 wherein the colored compound quenches blue, green or red light.

17. (Original) The method according to claim 2 or 4 wherein in step (a), a luciferase-mediated luminescence reaction is detected or determined.

18. (Original) The method according to claim 17 wherein the luciferase-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof.

19. (Original) The method according to claim 18 wherein the luciferase-mediated luminescence reaction is mediated by *Renilla reniformis* (sea pansy) luciferase or a functional equivalent thereof.

20. (Original) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof.

21. (Original) The method according to claim 20 wherein the second enzyme-mediated luminescence reaction is mediated by *Renilla reniformis* (sea pansy) luciferase or a functional equivalent thereof.

22. (Original) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is mediated by a luciferase.

23. (Original) The method according to claim 22 wherein the second enzyme-mediated luminescence reaction is mediated by *Photinus pyralis* (North American firefly) luciferase, *Pyrophorous plagiophthalamus* luciferase, or a functional equivalent thereof.

24. (Original) The method according to claim 1, 3 or 4 wherein one of the enzyme-mediated luminescence reactions detects the presence or amount of a substrate, enzyme or cofactor.

25. (Original) The method according to claim 1, 2, 3 or 4 wherein in step (a), a peroxidase-mediated luminescence reaction is detected or determined.

26. (Original) The method according to claim 25 wherein a horseradish peroxidase-mediated luminescence reaction is detected or determined.

27. (Original) The method according to claim 1, 2, 3 or 4 wherein in step (a), a phosphatase-mediated luminescence reaction is detected or determined.

28. (Original) The method according to claim 27 wherein alkaline phosphatase-mediated luminescence reaction is detected or determined.

29. (Original) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is a peroxidase-mediated luminescence reaction.

30. (Original) The method according to claim 29 wherein the second enzyme-mediated luminescence reaction is a horseradish peroxidase-mediated luminescence reaction.

31. (Original) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is a phosphatase-mediated luminescence reaction.

32. (Original) The method according to claim 31 wherein the second enzyme-mediated luminescence reaction is an alkaline phosphatase-mediated luminescence reaction.

33. (Original) The method according to claim 1, 3 or 4 wherein in step (a), a first luciferase-mediated luminescence reaction is detected or determined; and the second enzyme-mediated luminescence reaction is a second and distinct luciferase-mediated luminescence reaction.

34. (Original) The method according to claim 33 wherein in step (a), the first enzyme-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof; and the second enzyme-mediated luminescence reaction is mediated by a beetle luciferase or a functional equivalent thereof.

35. (Original) The method according to claim 34 wherein the second enzyme-mediated luminescence reaction is mediated by a *Photinus pyralis* or a *Pyrophorus plagiophthalamus* luciferase.

36. (Original) The method according to claim 34 wherein in step (a), the first enzyme-mediated luminescence reaction is mediated by *Renilla reniformis* luciferase.

37. (Original) The method according to claim 2 wherein the reaction detects the presence or amount of a substrate, enzyme or cofactor.

38. (Original) The method according to claim 1, 3 or 4 further comprising:

subsequent to detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction, quenching the second enzyme-mediated luminescence reaction by introducing a composition comprising at least one second quench reagent capable of quenching the second enzyme-mediated luminescence reaction.

39. (Original) The method of claim 38 wherein the at least one second quench reagent is capable of selectively quenching the second enzyme-mediated reaction.

40. (Original) The method of claim 1, 2 or 3 wherein the selective quench reagent quenches the first enzyme-mediated luminescence reaction by at least 35-fold.

41. (Original) The method of claim 1, 2 or 3 wherein more than one selective quench reagent is present in the composition.

42. (Original) The method of claim 41 wherein the selective quench reagents quench the first enzyme-mediated luminescence reaction by at least 100-fold.

43. (Original) An enzyme-mediated luminescence reaction assay kit comprising:

at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction, wherein the substrate is not a beetle luciferase substrate;

a suitable first container, the at least one functional enzyme substrate disposed therein;

a composition comprising at least one selective quench reagent which is a substrate analog inhibitor for the enzyme which mediates the luminescence reaction, a colored compound, or a nonionic detergent which is not Triton® X-100 or Tween® 20;

a suitable second container, the composition disposed therein; and

instructions for use.

44. (Original) An enzyme-mediated luminescence reaction assay kit comprising:

at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;

a suitable first container, the at least one functional enzyme substrate disposed therein;

a composition comprising at least one selective quench reagent for an anthozoan luciferase;

a suitable second container, the composition disposed therein; and

instructions for use.

45. (Original) The kit according to claim 43 or 44 wherein the selective quench reagent is a substrate analog inhibitor which is a protected coelenterazine.

46. (Original) The kit according to claim 43 or 44 wherein the selective quench reagent is a crown ether, glycol, or cyclodextran.

47. (Original) The kit according to claim 44 wherein the selective quench reagent is a nonionic detergent which is not Triton® X-100 or Tween® 20.

48. (Original) The kit according to claim 43 or 44 wherein the selective quench reagent is a yellow colored compound.

49. (Original) A dual reporter enzyme-mediated luminescence reaction assay kit comprising:

a first functional enzyme substrate for a molecule to be detected by a first enzyme-mediated luminescence reaction;

a suitable first container, the first functional enzyme substrate disposed therein;

a quench-and-activate composition comprising at least one selective quench reagent for an enzyme which mediates the first luminescence reaction and a second and distinct functional enzyme substrate corresponding to a second and distinct enzyme-mediated luminescence reaction, wherein the enzyme which mediates the first luminescence reaction is not a beetle luciferase;

a suitable second container, the quench-and-activate composition disposed therein; and

instructions for use.

50. (Original) A dual reporter enzyme-mediated luminescence reaction assay kit comprising:

a first functional enzyme substrate for a molecule to be detected by a first enzyme-mediated luminescence reaction, wherein the substrate is not a substrate for a beetle luciferase;

a suitable first container, the first functional enzyme substrate disposed therein;

a quench-and-activate composition comprising at least one selective quench reagents and a second and distinct functional enzyme substrate corresponding to a second and distinct enzyme-mediated luminescence reaction;

a suitable second container, the quench-and-activate composition disposed therein; and

instructions for use.

51. (Original) The kit according to claim 49 or 50 wherein the first functional enzyme substrate, and the second and distinct functional enzyme substrate, are luciferase substrates.

52. (Original) The kit according to claim 49 or 50 which comprises a nonionic detergent which is not Triton® X-100 or Tween® 20, a substrate analog inhibitor which is a protected coelenterazine, a yellow compound, or a combination thereof.

53. (Original) The kit according to claim 49 or 50 wherein the sequestering agent is a crown ether, glycol, or cyclodextran.

54. (Original) The kit according to claim 49 or 50 further comprising:

a second quench reagent capable of quenching photon emission from the second and distinct enzyme-mediated reaction; and

a suitable third container, the second quench reagent disposed therein.

55. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction; and

(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing at least one quench reagent to the luminescence reaction, wherein the quench reagent comprises a nonionic detergent that is not Triton® X-100 or Tween® 20, a substrate analog inhibitor for an anthozoan luciferase, a colored compound, or a combination thereof.

56. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction; and

(b) quenching the first enzyme-mediated luminescence reaction by introducing a composition comprising at least one quench reagent to the luminescence reaction, wherein the quench reagent comprises a nonionic detergent that is not Triton® X-100 or Tween® 20, a substrate analog inhibitor for an anthozoan luciferase, a colored compound, or a combination thereof.

57. (Original) A method to reduce or inhibit analyte-independent or analyte-dependent phosphorescence in an enzyme-mediated luminescence reaction, comprising:

(a) contacting a sample comprising an enzyme that mediates a luminescence reaction with a reaction mixture for the enzyme comprising a colored compound, which mixture does not comprise the enzyme, wherein the color of the compound is substantially the same as the light emitted in the luminescence reaction; and

(b) detecting or determining luminescence energy.

58. (Original) A method to reduce or inhibit analyte-independent or analyte-dependent phosphorescence in an enzyme-mediated luminescence reaction, comprising:

(a) contacting a sample comprising an enzyme that mediates a luminescence reaction and a colored compound with a reaction mixture for the enzyme, wherein the color of the compound is substantially the same as the light emitted in the luminescence reaction; and

(b) detecting or determining luminescence energy.

59. (Original) The method according to claim 57 or 58 further comprising detecting or determining luminescence energy in the reaction mixture prior to contacting the mixture with the sample.

60. (Original) The method according to claim 57 or 58 wherein the compound is a red, yellow, blue or green colored compound.

61. (Original) The method according to claim 57 or 58 wherein the enzyme is an anthozoan luciferase.

62. (Original) The method according to claim 57 or 58 wherein the enzyme is a beetle luciferase.

63. (Original) An enzyme-mediated luminescence reaction assay kit comprising:

at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;

a suitable first container, the at least one functional enzyme substrate disposed therein;

at least one colored compound;

a suitable second container, the at least one colored compound disposed therein; and

instructions for use,

wherein the color of the at least one compound is substantially the same as the light emitted by the enzyme-mediated luminescence reaction.

64. (Original) An enzyme-mediated luminescence reaction assay kit comprising:

at least one colored compound and at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;

a suitable first container, the at least one colored compound and the at least one functional enzyme substrate disposed therein; and

instructions for use,

wherein the color of the at least one compound is substantially the same as the light emitted by the enzyme-mediated luminescence reaction.

65. (Original) An enzyme-mediated luminescence reaction assay kit comprising:

a quench-and-activate composition comprising at least one selective quench reagent for an enzyme which mediates a luminescence reaction and a functional enzyme substrate for a molecule to be detected by a second and distinct enzyme-mediated luminescence reaction, wherein the enzyme which mediates the first luminescence reaction is not a beetle luciferase;

a suitable container, the quench-and-activate composition disposed therein; and

instructions for use.

66. (Original) The kit of claim 65 wherein the second enzyme-mediated luminescence reaction is a beetle luciferase-mediated luminescence reaction.

67. (Original) The kit of claim 65 wherein the first enzyme-mediated luminescence reaction is an anthozoan luciferase-mediated luminescence reaction.

68. (Original) The kit of claim 65 wherein the selective quench reagent is a nonionic detergent that is not Triton® X-100 or Tween® 20, a substrate analog inhibitor for an anthozoan luciferase, a colored compound, or a combination thereof.

69. (Original) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction which is not a beetle luciferase-mediated reaction; and

(b) introducing a composition capable of selectively quenching the first enzyme-mediated luminescence reaction and initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction, wherein the composition comprises at least one selective quench reagent which is a substrate analog inhibitor for the first enzyme, a nonionic detergent that is not Triton® X-100 or Tween® 20, or a colored compound; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.